

Measurement of the labile iron pool

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 An abbreviated version of this protocol was published in eLIFE in Feb 2021

Iron derived from autophagy-mediated ferritin degradation induces cardiomyocyte death and heart failure in mice

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Detailed protocol

Reagents

- Calcein-AM (Invitrogen: C1430)
- Pyridoxal isonicotinoyl hydrazine (PIH; Abcam: ab145871)
- MEM (glutamine- and phenol red-free, Gibco: 51200038) supplemented with 1x MEM non-essential amino acids solution (Gibco: 11140035), 100 µg/ml bovine serum albumin, insulin (10 mg/l)-transferrin (5.5 mg/l)-sodium selenite (6.7 µg/l) media supplement (ITS; Gibco: 41400045), 2 mM L-glutamine, 100 U/ml penicillin, and 100 g/ml streptomycin (Sigma-Aldrich: G6784).

Method

1. 2000 cardiomyocytes are seeded in 96 well plate (1 well = 200 µl).
2. Thirty min after seeding the cells, aspirate 150 µL medium, and then add 150 µL medium containing with or without 10 µM ferrostatin-1 (Sigma Aldrich: SML0583) for 30 min.
3. Thirty min later, aspirate 150 µL medium, and then add 150 µL medium with 10 or 20 µM erastin (Sigma Aldrich: E7781), 10 or 100 µM isoproterenol (Sigma Aldrich: I5627).
4. After stimulation, the cells are washed by medium three times (discard 150 µl medium) and incubated with 1 µM calcein-AM in 150 µL medium at 37°C for 10 minutes.
5. The cells are washed three times with the medium (discard 150 µl medium).
6. The fluorescence is measured using a fluorescence microplate reader.
7. Then, the cells are treated with 10 µM pyridoxal isonicotinoyl hydrazine (PIH; Abcam: ab145871) at 37°C for 10 minutes.
8. The cells are washed three times with the medium (discard 150 µl medium).
9. The fluorescence is measured again in a fluorescence microplate reader.
10. The changes in fluorescence (DF) upon PIH treatment is calculated for each sample.

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Ito, J. (2021). Measurement of the labile iron pool. Bio-protocol Preprint. bio-protocol.org/prep910.
2. Ito, J., Omiya, S., Rusu, M., Ueda, H., Murakawa, T., Tanada, Y., Abe, H., Nakahara, K., Asahi, M., Taneike, M., Nishida, K., Shah, A. M. and Otsu, K. (2021). Iron derived from autophagy-mediated ferritin degradation induces cardiomyocyte death and heart failure in mice. eLIFE. DOI: [10.7554/eLife.62174](https://doi.org/10.7554/eLife.62174)

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